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(12) **EUROPEAN PATENT APPLICATION**

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Declaration under Rule 28(4) EPC (expert solution)

(54) **Inhibition of phosphatidylinositol 3-kinase with wortmannin and analogs thereof.**

(57) Wortmannin and certain of its analogs are inhibitors of phosphatidylinositol 3-kinase. The compounds are particularly useful for inhibiting phosphatidylinositol 3-kinase in mammals and for treating phosphatidylinositol 3-kinase-dependent conditions, especially neoplasms, in mammals.

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B. Tank Fermentation of Culture A24603.1

In order to provide a larger volume of inoculum, 10 mL of incubated shake-flask medium, prepared as described in Section A, was used to inoculate 400 mL of a second-stage vegetative medium having the same composition as described above. This second-stage medium was incubated in a 2-L wide-mouth Erlenmeyer flask at 25° C for about 23 hours on a shaker orbiting in a two-inch (5.08 cm) circle at 250 rpm.

This second-stage medium (400 mL) was used to inoculate 115 L of sterile production medium having the following composition.

Production Medium	
Ingredient	Amount (g/L)
Glucose	25.0
Corn Starch	10.0
Lexein	10.0
Enzyme-hydrolyzed casein	4.0
Blackstrap molasses	5.0
$MgSO_4$ (anhydrous)	5.0
$CaCO_3$	2.0
Deionized H_2O	q.s. to 115 L

Unadjusted pH = 6.8; no adjustment.
Antifoam agent added: SAG 471[b] (0.2 gm/L).

[a] NZ Amine A (Sheffield Chemical Co., Norwich, NY).

[b] SAG 471 (Union Carbide, Sistersville, WV).

The inoculated production medium was allowed to ferment in a 115-L stirred fermentation tank for 4-5 days at a temperature of about 25° C. A dissolved oxygen level of about 45% of air saturation was maintained, as was a low rpm (180-330) in the stirred vessel.

Example 2

Isolation and Purification of Wortmannin

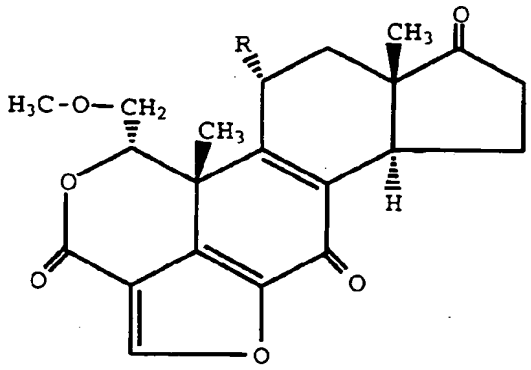
Fermentation broth from Example 1 was filtered through a ceramic filter (Membralox Systems, Illinois Water Treatment, Rockford, IL) to yield 175 L of filtrate containing wortmannin. The pH of the filtrate was adjusted to about 3.9 with 5N HCl. The filtrate was then eluted three times with one-half volumes of ethyl acetate to give a combined volume of 207 L which was concentrated to 6 L *in vacuo*.

The 6 L of ethyl acetate concentrate was further concentrated *in vacuo* to form a dark brown viscous oil to which 500 mL of methanol was added. The mixture was swirled until the resulting crystallization was complete, filtered, briefly washed with cold methanol and dried *in vacuo* to give 20.4 g of wortmannin.

The methanol supernatant was reconcentrated *in vacuo* to form a viscous oil, dissolved in 180 mL of chloroform and applied to a 12 x 20 cm column of Woelm Grade 62 silica in chloroform. 5.0 L of chloroform wash was concentrated *in vacuo* to form a brown oil which was then dissolved in 250 mL of warm methanol. The resulting crystals were collected after 18 hours, via filtration, giving 4.2 g of wortmannin. The crystallization procedure was repeated on the remaining supernatant, yielding an additional 1.9 g of wortmannin. The identity of wortmannin was confirmed by HPLC.

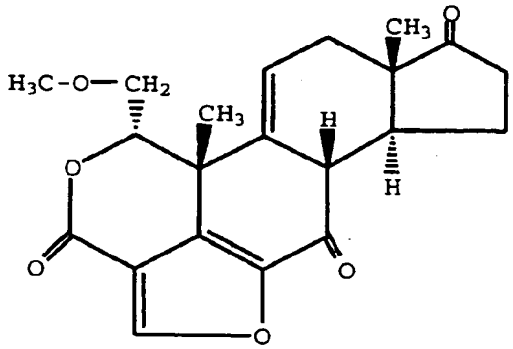
Claims

1. Use of a compound selected from the group consisting of



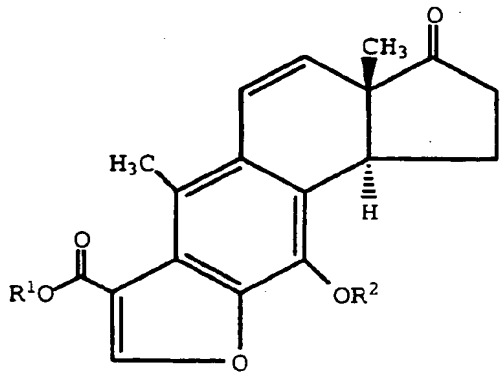
I

wherein R is H or acetoxy;



II ;

and



III

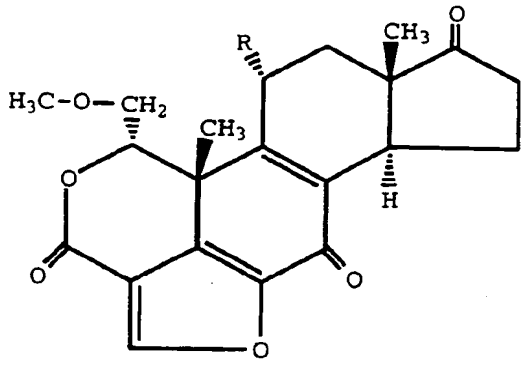
wherein

R^1 is H, methyl, or ethyl; and

R^2 is H or CH_3, for the manufacture of a medicament for inhibiting phosphatidylinositol 3-kinase in

mammals.

2. The use according to Claim 1 wherein said compound is a compound of formula I

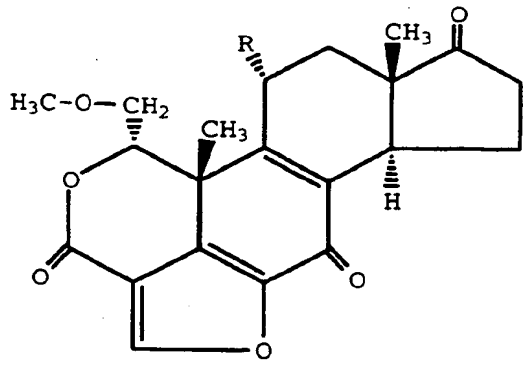


I

wherein R is H or acetoxy.

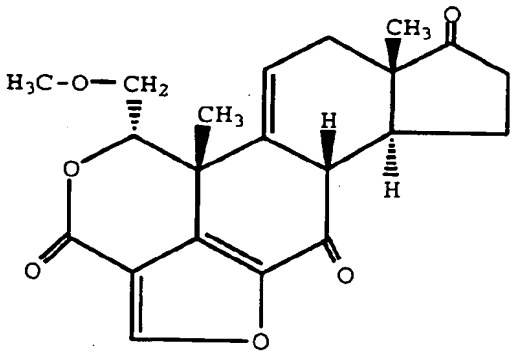
3. The use according to Claim 2 wherein said formula I compound is a compound wherein R is acetoxy.

4. Use of a compound selected from the group consisting of



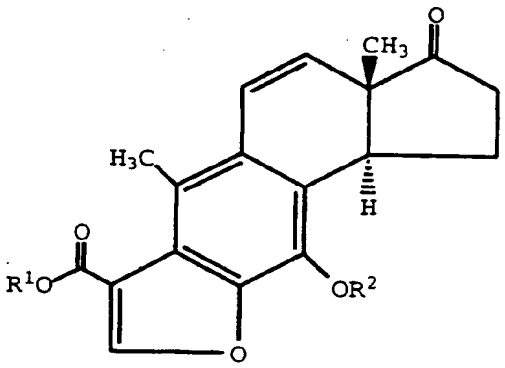
I

wherein R is H or acetoxy;



II ;

and



III

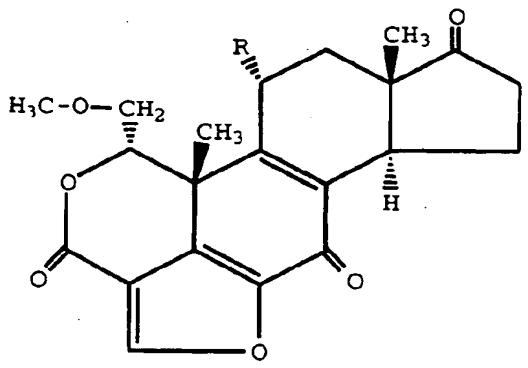
wherein

R^1 is H, methyl, or ethyl; and

R^2 is H or CH_3, for the manufacture of a medicament for treating phosphatidylinositol 3-kinase-dependent conditions in mammals.

5. The use according to Claim 4 wherein said phosphatidylinositol 3-kinase-dependent condition is a neoplasm.

6. The use according to Claim 5 wherein said compound is a compound of formula I

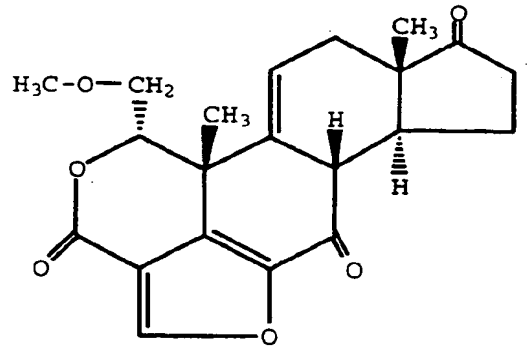


I

wherein R is H or acetoxy.

7. The use according to Claim 6 wherein said formula I compound is a compound wherein R is acetoxy.

8. The use according to Claim 4 wherein said compound is a compound of formula II



II.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 94 30 5192

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
P,X	BIOCHEM. J. vol. 296, no. 2 , December 1993 pages 297 - 301 A. ARCARO ET AL. 'Wortmannin is a potent phosphatidylinositol 3-kinase inhibitor: the role of phosphatidylinositol 3,4,5-triphosphate in neutrophil responses' * the whole document * ---	1-4	A61K31/365 A61K31/34
P,X	CANCER RES. vol. 54 , 1 May 1994 pages 2419 - 2432 G. POWIS ET AL. 'Wortmannin, a potent and selective inhibitor of phosphatidylinositol 3-kinase' * the whole document * ---	1-4	
P,X	DATABASE BIOSIS BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US Dialog Information Systems Access. no. 10242666 1994, J.A. PLUMB ET AL. 'Anti-tumor activity of a putative inhibitor of receptor-mediated phospholipase D activation' * abstract * ---	1-8	
X	& PROC. AM. ASSOC. CANCER RES. ANNU. MEETING vol. 34, no. 0 , May 1993 page 84 '84th Annual Meeting of the American Association for Cancer Research, Orlando, Florida, USA, May 19-22, 1993' --- -/--	1-8	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)
A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	28 October 1994	Foerster, W

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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European Patent Office

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Application Number
EP 94 30 5192

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y	BR. J. PHARMACOL. vol. 103, no. 1 , 1991 pages 1237 - 1241 R.W. BONSER ET AL. 'Demethoxyviridin and wortmannin block phospholipase C and D activation in the human neutrophil' * the whole document * ---	1-8	
Y	LABORATORY INVESTIGATION vol. 69, no. 1 , July 1993 pages 19 - 23 P.H. NACCACHE ET AL. 'Inhibition of tyrosine phosphorylation by wortmannin in human neutrophils' * the whole document * -----	1-8	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)

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